EFFECTS OF 4-NONYLPHENOL AND FORMULATIONS OF FIVE PESTICIDES: CYPERMETHRIN, DELTAMETHRIN, GLYPHOSATE, IMIDACLOPRID AND MANCOZEB ON GROWTH OF ATLANTIC SALMON (*Salmo salar* L.) DURING PARR-SMOLT TRANSFORMATION

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by

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ABSTRACT

Lyons, M., MacKeigan K., Fairchild, W.L. and Burridge, L.E. 2018. Effects of 4-nonylphenol and formulations of five pesticides: cypermethrin, deltamethrin, glyphosate, imidacloprid and mancozeb on growth of Atlantic salmon (Salmo salar L.) during parr-smolt transformation. Can. Tech. Rep. Fish. Aquat. Sci. 3265: v + 42 p.

This study has shown that short term, freshwater exposure of Atlantic salmon (*Salmo salar* L.) to some pesticide formulations during parr-smolt transformation (PST) caused significant reduction of specific growth rate of fish compared to the control fish over the course of a critical seawater growth period. Effects of 4-nonylphenol and formulations of five pesticides: cypermethrin, deltamethrin, glyphosate, imidacloprid and mancozeb, were tested. All the chemicals tested affected fish growth in seawater at either the July or September sample times. Other studies link poor growth near PST to reduced survival at sea and lower returns of adult salmon to their native streams. These results suggest that wild Atlantic salmon smolts may be affected by pesticides in rivers supporting sea-run salmon stocks. The consequences of reduced growth may be failure to survive or thrive at sea.

RÉSUMÉ

Lyons, M., MacKeigan K., Fairchild, W.L. and Burridge, L.E. 2018. Effects of 4-nonylphenol and formulations of five pesticides: cypermethrin, deltamethrin, glyphosate, imidacloprid and mancozeb on growth of Atlantic salmon (*Salmo salar* L.) during parr-smolt transformation. Can. Tech. Rep. Fish. Aquat. Sci. 3265: v + 42 p.

Cette étude a montré que l'exposition à court terme du saumon atlantique (*Salmo salar* L.) à quelques formulations de pesticides pendant la transformation tacon-saumoneau (TTS) entraînait une réduction significative du taux de croissance spécifique du poisson par rapport au poisson témoin au cours d'une période critique de croissance dans l'eau de mer. Les effets du 4-nonylphénol et des formulations de cinq pesticides: cyperméthrine, deltaméthrine, glyphosate, imidaclopride et mancozèbe ont été testés. Tous les produits chimiques testés ont affecté la croissance des poissons dans l'eau de mer aux périodes d'échantillonnage de juillet ou de septembre. D'autres études établissent un lien entre la faible croissance près de la TTS et la réduction de la survie en mer et le retour plus faible des saumons adultes dans leurs rivières natales. Ces résultats suggèrent que les saumoneaux du saumon de l'Atlantique sauvages pourraient être affectés par les pesticides dans les rivières qui supportent les stocks de saumon de mer. Les conséquences d'une croissance réduite peuvent inclure l'incapacité de survivre ou de prospérer en mer.

INTRODUCTION

The application of many current-use pesticides (CUPs) has increased with a transition away from use of older or banned pesticides that are persistent, bioaccumulative or toxic (Harris et al., 2008, Shelley et al., 2009). Many of the CUPs are considered to be of lesser environmental concern due to the perception of generally more favourable physico-chemical properties such as shorter environmental half-lives or decreased potential for bioaccumulation, both of which would be expected to contribute to a lower potential for toxicity in exposed organisms. However, increased toxicity of some newer pesticides to certain groups of organisms can actually increase total Toxic Units applied, even while total mass of pesticide applied declines (Hartwell, 2011). The concentrations of CUPs found in the environment, in most cases, are much lower than that expected to cause direct lethality in non-target aquatic species but there is still only limited information regarding their sub-lethal effects. Pesticides can be introduced into the aquatic environment by surface runoff, drift, leaching from the soil, accidental spill and atmospheric deposition (Shelley et al., 2009). In two decades spanning 1992 to 2011, the proportion of assessed streams and rivers in the U.S. with one or more pesticide concentrations in water exceeding an aquatic life benchmark were very similar, ranging from 45 to 90% (Stone et al., 2014). In Canada, pesticide registration is performed by Health Canada and the risk assessments associated with pesticide use remain based, for the most part, on threshold data derived from studies with the active ingredient and rarely using commercial pesticide formulations.

Atlantic salmon (*Salmo salar* L.) are an anadromous fish species that undergo distinct physiological and morphological transformations prior to their seaward migration from their freshwater rearing habitat (Hoar, 1988). The seawater transformation process is known as smoltification or parr-smolt transformation (PST) and the activity of thyroid hormones, prolactin, growth hormone, corticosteroids and possibly gonadal steroids triggers the transition of fresh water adapted parr into sea-going smolts (Keen et al., 2005). Temperature and photoperiod are the two environmental cues that interact and have impact on the neuroendocrine system that regulates physiological changes. There is a limited period of usually a few weeks time each spring, during which smolts are best able to go to sea, a both physiological and ecological "smolt window" (McCormick et al., 1998). Often the rivers and estuaries that smolts use as migration corridors are impacted by pollution (McCormick et al., 1998; Ross et al., 2013). Smolt development has been shown to be adversely affected by acidity and aluminum, pollutants and improper husbandry conditions and is often more sensitive than other life stages (McCormick et al., 1998; McCormick et al., 2002; McCormick et al., 2009; McCormick et al., 2012; Thorstad et al., 2012)

Recent research has demonstrated that freshwater and marine environments cannot be considered in isolation and that conditions within the freshwater zone experienced by Atlantic salmon may be critical to their subsequent survival in the sea (Fairchild et al., 2002; Kroglund et al., 2008; McCormick et al., 2009; Thorstad et al.,

2013). Exposure of juvenile salmon to a range of sub-lethal contaminants, such as pesticides and endocrine-disrupting chemicals (EDCs), may operate to reduce survival in fish that have migrated to sea (Madsen et al., 2004). 4-Nonylphenol (4-NP) is an EDC capable of mimicking the action of 17 β-estradiol (E_2). It has been hypothesized that 4-NP as a solvent in a pesticide formulation was linked to historical declines in Canadian Atlantic salmon populations, with effects being related to exposure during later stages of PST (Fairchild et al., 1999). Experiments investigating the effects of water-borne concentrations of E_2 and 4-NP on PST indicated that a portion of the Atlantic salmon smolts treated with E_2 and 4-NP experienced compromised growth (Arsenault et al., 2004).

Fish respond to stressors by eliciting a generalized physiological stress response, which is characterized by an increase in stress hormones and consequent changes that help maintain the animal's normal or homeostatic state. These physiological alterations are grouped as primary responses, which include hormonal changes, and secondary responses, which include changes in metabolites, blood ions and hematology (Iwama et al., 1999; Barton, 2000; Iwama et al., 2004). The tertiary response represents whole-animal and population level changes associated with stress. Exposure to stressors, depending on the intensity and duration can lead to decreases in growth, disease resistance, reproductive success, smolting, swimming performance and other characteristics of the whole animal or population (Iwama et al., 2004).

Change in weight (mass) is the most commonly used assessment for growth performance. In general, when growth rate is exponential, as it usually is over intervals of a year or less, growth can be expressed as an instantaneous growth rate or as a percentage of instantaneous growth called specific growth rate (Busacker et al., 1990).

Biochemical endpoints can provide information on the sublethal, cellular effects of stressors in a particular species of interest and have the potential to be applied as sensitive biomarkers in field studies to monitor fish health (Eder et al., 2009). For over 40 years, researchers have been studying the function of the ubiquitous stress or heat shock proteins that protect cells from the potentially damaging effects of a wide variety of stresses including exposure to environmental contaminants. Stress protein synthesis is induced by stressors of major environmental concern, including various pesticides that differ in their chemical and physical characteristics (Sanders, 1993). Hsp 70 has the largest specific activity of the stress proteins, and may be easier to detect. In some circumstances the induction of hsp70 shows a clear response where "present" and "absent" may serve as a biomarker (Lewis et al., 1999).

Growth hormone and cortisol interact in teleosts to increase salinity tolerance and the underlying physiological changes such as gill Na^+, K^+-ATPase activity. Na^+, K^+-ATPase is a transport enzyme that plays a central role in the salt-secretory function of chloride cells. Regulation of chloride cells and Na^+, K^+-ATPase is critical during movement of fish between fresh water and sea water. Since the neuroendocrine system is

the primary link between a changing environment and physiological adaptation, the hormonal control of Na^+, K^+-ATPase is critical (McCormick, 1995). Gill Na^+, K^+-ATPase activity is a widely used biomarker for ion regulatory changes. Elevation of circulating cortisol is also considered a stress response in salmonids (Barton, 2000).

The objectives of the present study were two-fold. The first objective was to examine the effects of water-borne formulated CUPs on growth performance of Atlantic salmon smolts. The second objective was to examine the sub-lethal cellular effects of formulated CUPs on Atlantic salmon smolts using biochemical endpoints. We exposed the smolts to environmentally relevant, pulse doses of the CUPs. Five CUPs were selected for these purposes- two synthetic pyrethroid insecticides, cypermethrin and deltamethrin; one neonicotinoid insecticide - imidacloprid; one fungicide, the carbamate, mancozeb and one herbicide, glyphosate. 4-Nonylphenol, a putative EDC that has been shown to impair growth in smolts was used as a positive control. Except for the positive control all CUPs were applied in commercially available formulations.

MATERIALS AND METHODS

FISH

Atlantic salmon smolts (Saint John River, New Brunswick, stock) were obtained from the Department of Fisheries and Oceans (DFO), Mactaquac Biodiversity Center, Mactaquac, New Brunswick, in March of each year (2007-2010) and brought to the DFO

St. Andrews Biological Station, St. Andrews, New Brunswick. Smolts (30-80 g) were anaesthetized in 50 ppm MS222 (Tricaine Methane Sulphonate) and individually tagged with passive integrated transponder (PIT) tags (AVID Canada, Calgary, Alberta). Fork length and weight of fish were recorded. Fish were randomly distributed into fiberglass tanks (400 L, 40 fish per tank) and allowed to acclimate in dechlorinated St. Andrews, New Brunswick municipal water at ambient temperature prior to treatments. The flow rate was maintained at approximately 5 L/min and photoperiod was regulated to simulate natural photoperiod. Except on treatment and sampling days, the fish were fed a 2.5 or 3 mm dry pellet diet from Skretting, Bayside, New Brunswick. Protocols for all experiments were approved by DFO regional animal care committee.

CHEMICALS

The five current use pesticides that were tested, were purchased as formulations commercially available in Canada at the time (2007-2010). RIPCORD™ 400 EC (BASF Canada Inc, Mississauga, Ontario) is an insecticide formulation with active ingredient cypermethrin. Decis® 5 EC (Bayer CropScience Inc, Calgary, Alberta) is an insecticide formulation with active ingredient deltamethrin, Roundup WeatherMax® (Monsanto Canada Inc, Winnipeg, Manitoba) is a herbicide formulation with active ingredient glyphosate. Admire® (Bayer CropScience Inc, Calgary, Alberta) is an insecticide formulation with active ingredient imidacloprid. Manzate® 200 DF (Dupont Canada Co,

Mississauga, Ontario) is a fungicide formulation with active ingredient mancozeb. P-Nonylphenol (Eastman Kodak Co, Rochester, New York) was used as a positive control.

TREATMENTS

In May of each year, the tanks were randomly assigned to be one of two replicate tanks, of one of the treatments (i.e. control, 4-NP, three concentrations of pesticide). The flow of dechlorinated water into each tank was adjusted to 4 L/min. The fish were exposed to water-borne pesticide, 4-NP or no treatment (controls) in freshwater. The exposures took place when the freshwater temperature reached approximately 7°C. The 4-NP was dissolved in 10% ethanol/dechlorinated water. Two replicate tanks were treated with an environmentally-relevant (Fairchild et al., 1999) nominal concentration of 4-NP (20 $\mu g \cdot L^{-1}$). The pesticide formulations were dissolved in dechlorinated water and replicate tanks were treated with three concentrations. The 4-NP treatments were delivered continuously in two 24 h pulses on day 1 and 7. The pesticide treatments were delivered continuously in two 6 h pulses on day 1 and 7. The treatments were started by adding the appropriate amount of test solution required to bring each tank up to the desired concentration. Mariott bottles of the test solutions were calibrated to gravity-feed the treatment solutions at a flow rate of 1 mL/ min directly into the incoming water supply of each treatment tank to ensure complete mixing. Beginning 2-3 weeks after the onset of treatments, the smolts were gradually acclimated to filtered sea water (Brandy

Cove, St. Andrews, New Brunswick) over a period of 3-4 days. Seawater flow rates were maintained at approximately 5 L/ min.

SAMPLING

Pre-treatment sampling (one fish per tank) occurred on the day before the first treatment. Post-treatment sampling (four or five fish per tank) occurred for most treatments in May/June and for all treatments on two occasions, July and November (five fish per tank). Fish were stunned with a sharp blow to the head and blood was collected from the caudal vein with a heparinized disposable syringe and centrifuged at 3500 x g for 10 minutes at 4°C. Plasma was aliquoted and stored at -80°C. Gill, liver, muscle and brain tissue were taken and stored for biochemical analyses. Tissues were flash frozen in liquid nitrogen and stored at -80°C. Length, weight, liver weight, blood volume and sex were recorded for each fish. After the July sampling, length and weight were recorded for all remaining fish in treatment tanks and an equal number of fish from each tank were randomly assigned to one of two large tanks (3000 L) for long-term seawater grow-out. In September length and weight were recorded for all fish. After the November sampling, remaining fish were sacrificed with an overdose of anaesthetic (MS-222) and length, weight and sex were recorded. Sampling protocols for these experiments were prepared according to the guidelines of the Canada Council for Animal Care, and were approved by the (DFO) Regional Animal Care Committee.

HEAT SHOCK PROTEIN DETERMINATION

Heat shock protein 70 analysis was performed on liver samples according to Rendell et al. (2006). Briefly, ground liver was added to 15 µl homogenization buffer per mg of tissue to extract soluble protein. Samples were homogenized and centrifuged at 14000 x g for 10 minutes in a Sorvall RC6 Plus centrifuge at 4°C. The supernatants were stored at -80°C after being aliquoted into two microcentrifuge tubes, one for total protein quantification and one for heat shock protein quantification.

Liver tissue supernatants were assayed for total protein concentration using the Bio-Rad DC Protein Assay for microtitre plates based on the Lowry method. The supernatants were diluted 1:100 with homogenization buffer for total protein analysis. Standards (BSA) and samples were read at 750 nm on a BioTek Powerwave XS 96 well plate reader. Once total protein levels in the liver samples had been determined, liver supernatants were diluted in 2 x SDS reducing buffer (50 % of total volume), 50 mmol/ L DTT (10 % of total volume) and deionized water and heated at 95°C for 4 minutes. The dilution of samples was required so that 15 µg of protein could be loaded into each well of a 10 % Tris-HCl Pre-Cast Criterion gel (Bio-Rad). A purified Chinook salmon hsp70 (SPP-763) protein standard (Stressgen) and one sample from a heat-shocked salmon were loaded onto each gel to allow direct comparison among gels. SDS-PAGE and immunoblotting were run using a Criterion cell and a Criterion Blotter apparatus (Bio-Rad). Primary antibody used was rabbit anti-salmonid hsp70 (AS05 061, Agrisera) and

the secondary antibody used was goat anti-rabbit (SAB-300, Stressgen). Protein detection was performed using the ECL Advance Chemiluminescent Western Blotting Detection kit (Amersham Pharmacia Biotech) and protein bands were visualized using a Molecular Imager- ChemDoc XRS (Bio-Rad). Band density was determined using Quantity One 1-D Analysis Software, v4.6.4 (Bio-Rad).

NA^+, K^+-ATPASE DETERMINATION

Salmon gill samples (2007) were collected, homogenized, extracted, and analyzed using the microplate (BioTek Powerwave XS plate reader) methodology outlined by McCormick (1993). Due to the difficulties in collecting consistent gill samples, resulting in multiple extract dilutions for protein analysis, the tissue collection and extraction technique for subsequent years was a modification of an extraction proposed by Zaugg (1982). Protein levels in the same samples and standards (BSA) were quantified by UV absorbance at 600 nm on a microplate using a Bio-Rad commercial assay reagent based on the Bradford method.

The tabulation of the final Na^+, K^+-ATPase values was based on the methods of Bolly et al. (2007). The results were expressed as micromoles of ADP per milligram of protein per hour.

AChE DETERMINATION

Brain tissue collected from fish exposed to pesticides that act by inhibiting acetylcholinesterase activity was analysed to determine enzyme activity according to Ellman et al. (1961), modified by Zinkl et al. (1987). Enzyme activities were determined at room temperature using a 1:2 brain homogenate in 0.1 M Tris buffer, pH 8. Acetylcholinesterase standard and sample absorbances were read at 412 nm on a Cary 300 Bio UV/VIS spectrophotometer. Total protein measurements for samples and standards (HSA and gamma-globulins) were made on a Cary 300 Bio UV/VIS spectrophotometer using a modification of the Lowry method by Miller (1959). The results were expressed as micromoles of AChE per milligram of protein per minute.

SPECIFIC GROWTH RATES

The specific growth rates based on weight (SGRW) were calculated for the time period between March PIT tagging date and July, September or November date where all available fish were measured. The SGRWs were calculated using the following formula: $SGRW = ((\log_e Y_2 - \log_e Y_1)/ (t_2 - t_1)) * 100$ where Y_2 is the weight in July, September or November, Y_1 is the weight in March, t_2 is the day of year in July, September or November, t_1 is the day of year in March.

WATER ANALYSES

Water samples (150 ml in high density polyethylene (HDPE) containers) from control and glyphosate treatment tanks were taken at T=2.5 h during the 6 h treatment (n=4). A blank water sample was taken from the dechlorinated water line. Glyphosate water samples were analysed at Natural Resources Canada, Great Lakes Forestry Centre, Sault Ste. Marie, Ontario, Canada, using methodology described by Struger et al. (2008).

Water samples (900 ml in IChem 200 amber bottles) from control (n=2) and mancozeb (n=4) treatment tanks were taken at T=4 h during the 6 h treatment. A blank water sample was taken from the dechlorinated water line. All were preserved with 6 N HCl until pH< 2 and refrigerated at ~ 2°C. Mancozeb water samples were analyzed according to Canadian Food Inspection Agency (CFIA, 2002) methods at Environment and Climate Change Canada, Moncton, New Brunswick, Canada (Art Cook (retired), Environment and Climate Change Canada, Moncton, New Brunswick, Personal Communication).

Water samples (900 ml in IChem 200 amber bottles) from control (n=2) and imidacloprid (n=4), deltamethrin (n=4) and cypermethrin (n=4) treatment tanks were taken at T=1-3 h during the 6 h treatment. A blank water sample was taken from the dechlorinated water line. All samples were preserved with 45 ml of dichloromethane and refrigerated at about 2°C. Imidacloprid, deltamethrin and cypermethrin water samples

were sent to Environment and Climate Change Canada, Moncton, New Brunswick, Canada, for analysis. The pyrethroid pesticides, deltamethrin and cypermethrin were analyzed based on method USEPA 8081A (Anonymous, 2011). Imidacloprid was analysed according to Baskaran et al. (1997).

Water samples (500 ml in IChem 200 amber bottles) from control (n=7) and nonylphenol (n=11) treatment tanks were taken at T=0 h, 3 h and 24 h during the 24 h treatment. A blank water sample was taken from the dechlorinated water line. All were preserved with 6 N HCl until pH< 2 and refrigerated at ~ 2°C. Nonylphenol water samples were analyzed at Fisheries and Oceans Canada, Institute of Ocean Sciences, Ocean Sciences Division, Sidney, British Columbia, Canada. Analyses were conducted according to Yoshida et al. (2007) with modification: specifically, no solid phase extraction was conducted and large volume injection (LVI) of 30 or 100 uL when 10 uL injection did not provided the necessary limit of detection (Andrew Ross, Fisheries and Oceans Canada, Sidney, BC, Personal Communication).

STATISTICAL ANALYSIS

Weights and specific growth rates based on weight (SGRW) were used for statistical analysis. A non-parametric one-way analysis of variance test (NPAR-1-ANOVA) followed by a Dunnett's multiple comparison test was used to compare the mean weights and SGRWs of the control smolts with those of 4-NP and pesticide-treated

smolts. The data sets used for the 2007 weight and SGRW analysis comprised of all controls, glyphosate and mancozeb treated smolts available in July (n = 55 – 71), September (n = 42 – 59) and November (n = 14 – 15). The data sets used for the 2008 weight and SGRW analysis comprised of all controls, 4-NP and imidacloprid treated smolts available in July (n = 29 – 66), September (n = 24 – 54) and November (n = 24 – 27). The data sets used for the 2009 weight and SGRW analysis comprised of all controls, 4-NP and deltamethrin treated smolts available in July (n = 53 - 58), September (n = 43 - 48) and November (n = 21 – 26). The data sets used for the 2010 weight and SGRW analysis comprised of all controls, 4-NP and cypermethrin treated smolts available in July (n = 38 - 54), September (n = 25 - 43) and November (n = 19 - 22). Similar to Arsenault et al. (2004) we applied Kolmogorov-Smirnov tests (KS test) to compare the frequency distributions of weight and SGRW. SPSS SAS version 17.0 (SAS Institute Inc., Cary, NC, USA) was used to conduct all of the statistical analyses.

RESULTS

GROWTH

A summary of comparisons of mean SGRWs between control and pesticide treatments shows there was significant difference between some treatments and controls. Comparisons between the mean SGRWs of control and treated smolts showed that SGRWs were different in July, for imidacloprid at both 90 mg/L and 10 µg/L, and for

glyphosate at both 650 µg/L and 65 µg/L. In September, cypermethrin at 0.75 µg/L, deltamethrin at 0.59 µg/L, and glyphosate at 650 µg/L, showed significant effects in comparison to controls. By November, there were no significant differences between mean SGRWs of control and any pesticide treated fish. (Dunnett's, $P < 0.05$). (Table 1 and Fig. 1a-c).

A summary of comparisons of frequency distributions of control and pesticide treatment SGRWs shows there was significant difference between some treatments and controls (Table 2). July SGRW frequency distributions (Figs. 2, 5, 6 &7) and frequency histograms (Figs. 8, 9, 10 & 11) for cypermethrin, imidacloprid, glyphosate and mancozeb treated smolts show how SGRWs of the treated smolts were affected relative to the controls. July SGRW frequency distributions of cypermethrin (0.75 µg/L), imidacloprid (90 mg/L and 8.6 mg/L), glyphosate (3000 µg/L and 650 µg/L) and mancozeb (50 µg/L) treated smolts were significantly different from controls ($P < 0.05$) (KS test). July SGRW frequency distributions of imidacloprid (10 µg/L) and glyphosate (65 µg/L) treated smolts were significantly different from controls ($P < 0.005$) (KS test). September SGRW frequency distributions of imidacloprid (10 µg/L), glyphosate (650 µg/L), cypermethrin (0.15 µg/L) (not shown) and deltamethrin (59 ng/L and 0.59 µg/L) (not shown) treated smolts were significantly different from controls ($P < 0.05$) (KS test) (Fig. 3). September SGRW frequency distributions of cypermethrin (0.75 µg/L) treated smolts were significantly different from controls ($P < 0.005$) (KS test). In November,

only the imidacloprid (90 mg/L) treated smolts SGRW frequency distributions were significantly different from controls ($P < 0.05$) (KS test) (Fig. 4).

July 2008 SGRW frequency distributions of NP (20µg/L) treated smolts were significantly different from controls ($P < 0.005$) (KS test) (Fig. 2). September 2008 and 2010 SGRW frequency distributions of NP (20µg/L) treated smolts were significantly different from controls ($P < 0.05$) (KS test) (Fig. 3). September 2010 mean SGRW of NP (20µg/L) treated smolts were less than controls ($P = 0.051$) (Dunnett's). July and September 2009 and July 2010 mean SGRWs of NP treated fish were less than controls, but the differences were not significant.

HSP 70

Post treatment (1 to 3 days) liver samples from May and June were analysed for hsp70 induction. Exposure to 6 h pulse sublethal levels of cypermethrin, deltamethrin, glyphosate, imidacloprid and mancozeb did not induce hsp70 in Atlantic salmon liver. Under control conditions there was no hsp70 detected in the liver. Hsp70 was detected in samples of liver from heat shocked Atlantic salmon that were loaded onto each gel as positive controls.

GILL NA^+, K^+-ATPASE

Post treatment gill samples from July were analysed for Na^+, K^+-ATPase activity. Mancozeb (150 µg/ L and 1500 µg/ L) treated fish had significantly decreased mean Na^+, K^+-ATPase activity compared to control fish. Comparisons between control and glyphosate treatments (3000µg/L , 650 µg/L and 65µg/L) and mancozeb (50 µg/L) mean Na^+, K^+-ATPase activity showed decreases in treated fish but the differences were not significant (Fig. 12) . Imidacloprid treated fish showed increased mean Na^+, K^+-ATPase activity compared to controls but the differences were not significant (Fig. 13) (Dunnett's, $P < 0.05$). Post treatment gill samples from June were analysed for Na^+, K^+-ATPase activity in deltamethrin, cypermethrin and NP treated smolts. All deltamethrin and cypermethrin treated fish showed increased mean Na^+, K^+-ATPase activity compared to controls but the differences were not significant (Dunnett's, $P < 0.05$). There were no significant differences between mean Na^+, K^+-ATPase activity in the June and July samples analyzed for NP treated smolts.

BRAIN AChE

Post treatment brain tissues were analysed for AChE activity in mancozeb treated fish. Mancozeb (150 µg/ L) treated fish had significantly different mean AChE activity one day post second treatment compared to controls {mancozeb 150 µg/L (9.30 µmol/min/mg tissue ± 0.232, n = 8), controls (11.07 µmol/min/mg tissue ± 0.802, n = 8)}.

Comparisons between control and mancozeb (50 µg/L and 1500 µg/L) mean AChE activity showed decreases in treated fish but the differences were not significant (Dunnett's, $P < 0.05$) (not shown). Comparisons of AChE activity in brain tissue sampled 1 day post first treatment and 20 days post first treatment showed no significant difference between mancozeb treated fish and controls (data not shown).

WATER ANALYSIS

Measured water concentrations for pesticide and NP treatments are shown in Table 3. These measured concentrations were generally lower than nominal concentrations. Lower measured concentrations are probably due to uptake by tubing, glassware, the surface of the tanks and the fish. Control tank water samples analysed did not show any detectable levels of pesticide. Results or imidacloprid water sample analysis are not available because of problems with the methodology at the contract laboratory.

DISCUSSION

In this study, SGRW frequency distributions of some pesticide treated smolts and controls were significantly different. Of particular interest are the treatments at low, environmentally relevant concentrations - deltamethrin 59 ng/L and 0.59 µg/L (September), cypermethrin 0.15 µg/L (September), imidacloprid 10 µg/L (July and September), glyphosate 65 µg/L (July) and mancozeb 50 µg/L (July), that affected fish

size. Some salmon exhibited poor seawater growth after short term exposure to pesticides during PST. Exposure to low levels of pesticides in their native rivers during the smolt runs to sea may be a factor responsible for decline in wild Atlantic salmon adult returns (Arsenault et al., 2004). Smaller fish in the wild soon after the PST may be at greater risk to decreases in disease resistance, reproductive success, smolting and swimming performance (Iwama et al., 2004). Baldwin et al. (2009) used models to show that environmentally realistic pesticide exposures may limit the recovery potential of salmon populations via delayed reductions in growth and survival. They suggested that toxicological linkages across biological scales should be established to identify which chemicals in salmon habitats should be a priority for toxic reduction strategies. All the exposures in the present study were to single pesticide formulations, whereas in the field, realistic exposure scenarios would include mixtures of more than one formulated product.

Deltamethrin has been detected in Canadian freshwater at concentrations of 10 ng/L to 24 µg/L (CCME, 1999a). Cypermethrin has been detected in surface water at a maximum concentration of 0.38 µg/L at the mouth of the Don River in Ontario (Struger and Fletcher, 2007). Imidacloprid has been detected in Prince Edward Island potato field runoff following rainfall events in the range of 0.5 µg/L to 11.9 µg/L (CCME, 2007). Mancozeb, as total dithiocarbamate, has been detected in field runoff at 1260 µg/L and in a river with a fish kill at 131 µg/L (Ken Doe (retired), Environment and Climate Change Canada, Moncton, NB, Personal Communication). Glyphosate has been detected in

Canadian freshwater in the range of 1.5 µg/L, as a result of direct overspraying, to 5153 µg/L in runoff water, where two to four times the recommended application rate was applied (CCME, 1999b). Glyphosate has a water quality guideline for the protection of aquatic life (interim) value of 65 µg/L (CCME, 1999b). Our study found growth effects on Atlantic salmon at this concentration ($P<0.005$) (Dunnett's).

All five products that affected growth at environmentally relevant concentrations have different modes of action. Deltamethrin and cypermethrin are non-systemic insecticides with contact and stomach action. They are pyrethroids that prevent the sodium channels from functioning so that no transmission of nerve impulses can take place. Imidacloprid is a systemic insecticide used to control sucking insects with contact and stomach action. It acts on the central nervous system, causing blockage of postsynaptic nicotinergic acetylcholine receptors. Glyphosate is a non-selective herbicide, absorbed by foliage, with rapid translocation throughout the plant. It inhibits 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), an enzyme of the aromatic acid biosynthetic pathway (Tomlin, 1997). Mancozeb is a fungicide with protective action. Mancozeb is a broad-spectrum fungicide belonging to a chemical class of polymeric dithiocarbamates and a group classified as ethylene-bis-dithiocarbamate (EBDC) fungicides. They are non-systemic, contact fungicides with preventive activity. The EBDC mancozeb can be metabolized to ethylenethiourea (ETU) which is of toxicological concern due to ETU's carcinogenicity, teratogenicity, and anti-thyroid properties

(Tomlin, 1997; U.S. EPA, 2007). Carbamate (CB) pesticides bind AChE but the bond is reversible and relatively short-lived. Fish can recover from exposure to CBs on a timescale of a few hours (Ferrari et al., 2004).

Levels of the inducible hsp70 are barely detectable in rainbow trout (*Oncorhynchus mykiss*) liver under control conditions but increase significantly after a 1 hour heat shock at 25°C and further increase following 24 h of recovery at 13°C (Rendell et al., 2006). Hsp70 is not detected in Atlantic salmon smolt liver under control conditions but is induced after a 15 minute heat shock at 26°C (DuBeau et al., 1998). Hsp70 is constitutively expressed in Chinook salmon (*Oncorhynchus tshawytscha*) liver and a 96 hour exposure to the insecticide, chlorpyrifos significantly increases that expression (Eder et al., 2009). Ceyhun et al. (2010) detected that deltamethrin increased the gene expression of hsp70 in rainbow trout muscle tissue in as little as 6 hours. The 6 h pulse dose exposures with pesticides in the present study failed to induce a hsp70 response in liver of Atlantic salmon.

Several studies with Atlantic salmon link exposure of the pesticide atrazine either alone or in combination with 4-NP to reduced gill ATPase activity (Moore et al., 2003; Waring and Moore, 2004; Moore et al., 2007; Nieves-Puigdoller et al., 2007; Moore et al., 2008). Li et al. (2010) linked a long term exposure of rainbow trout to the fungicide, propiconazole to an inhibition of Na^+, K^+-ATPase activity in brain. Suvetha et al. (2010) found a long term sublethal exposure of freshwater carp, *Cyprinus carpio* to

cypermethrin decreased gill Na^+, K^+-ATPase activity. Deltamethrin inhibited Na^+, K^+-ATPase activity in gill and heart tissue of the freshwater fish, *Ancistrus multispinis* (Silva de Assis et al., 2009). All pulse dose exposures of mancozeb and glyphosate in this study inhibited Na^+, K^+-ATPase activity in brain but significant differences from controls were only seen in brain tissue from mancozeb (150µg/L and 1500 µg/L) treated salmon.

Brain AChE activity decreased in Atlantic salmon parr after exposure to the OP fenitrothion (Morgan et al., 1990). Brain and muscular ChE were inhibited in juvenile rainbow trout exposed to the OP azinphos methyl and the CB carbaryl (Ferrari et al., 2007). Juvenile coho salmon (*Oncorhynchus kisutch*) exposed to five individual organophosphate and carbamate pesticides had concentration dependent decreases in brain AChE activity (Laetz et al., 2009). The CB fungicide mancozeb significantly increased brain AChE in coho salmon (Jarrard et al., 2004). In the present study all mancozeb treated fish had lower mean AChE activity one day post second treatment compared to controls but only the mancozeb (150 µg/ L) treated fish had mean AChE activity significantly different from controls. Comparisons of AChE activity in brain tissue sampled 1 day post first treatment and 20 days post first treatment showed no significant difference between mancozeb treated fish and controls. For salmonids in particular, acetylcholinesterase insecticides have been shown to disrupt several behaviours such as swimming, feeding and predator avoidance (Labenia et al., 2007; Baldwin et al., 2009). Feeding and energy homeostasis in vertebrates appears to be

regulated by specific regions of the brain that produce peptides that either increase (orexigenic factors) or decrease (anorexigenic factors) feeding (Pérez-Casanova et al., 2010).

CONCLUSION

Our study has shown that short term, freshwater exposure of Atlantic salmon to some pesticides during the PST caused a significant reduction of specific growth rate of fish compared to the control fish over the course of a critical seawater growth period. Poor growth near the PST has been linked to reduced survival at sea and fewer returns of adult salmon to their native streams. All of the chemicals tested affected fish growth at either the July or September sample times. It remains unclear if the growth response observed in Atlantic salmon smolts is specific to the chemical being tested or is a generalized response to stress, in this case chemical stress.

None of the compounds studied affected growth at all sampling times. The rate of growth picked up after an initial slow-down. Our frequency distribution work shows that the shift to smaller fish is evident in July and September but by the November sampling a shift to smaller fish is only evident for the highest treatment concentration of imidacloprid. Smaller fish in the wild soon after the PST may be at greater risk to decreases in disease resistance, reproductive success, smolting and swimming performance (Iwama et al., 2004). Baldwin et al. (2009) used models to show that

environmentally realistic pesticide exposures may limit the recovery potential of salmon populations via delayed reductions in growth and survival.

The negative effects on growth observed at environmentally relevant concentrations of deltamethrin, cypermethrin, imidacloprid, glyphosate and mancozeb are ecologically significant. These results suggest that wild Atlantic salmon smolts may be affected by pesticide runoff into rivers supporting sea-run salmon stocks. The consequences of reduced growth may be failure to survive or thrive at sea. The growth effects observed on fish exposed to glyphosate at a concentration equal to the freshwater interim guideline value of 65 µg/L evoke particular concern for the survival of wild Atlantic salmon smolts. The negative effects on growth after short-term pulse doses of waterborne glyphosate may be used to re-evaluate risk.

While this work shows effects when salmon smolts are exposed in freshwater two of the products, deltamethrin and cypermethrin are also applied to combat infestations of parasites on farmed Atlantic salmon (Burridge et al., 2010). It would be interesting to look at effects of treatments on growth of farmed smolts and on the potential to affect wild smolts on their spring migration.

The pesticides used in this study were commercial formulations that contain other products to enhance the efficacy of the pesticides. These products and the possible degradation products of the pesticides may play a role in the negative growth affects

seen. Exposure of Atlantic salmon to 4-nonylphenol during the PST always resulted in reduced growth compared to control fish. However, the reduction in growth was not always statistically significant. Arsenault et al. (2004) reported a significant effect of NP on Atlantic salmon smolts treated in the same experimental system used in this study.

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REFERENCES

Anonymous. 2011. Environmental Protection Agency Waters - Hazardous Wastes Tests Methods - SW846 On-line. www.epa.gov/osw/hazard/testmwethods/sw846/online

Arsenault, J.T.M., Fairchild, W.L., MacLatchy, D.L., Burridge, L., Haya, K. and Brown, S.B. 2004. Effects of water-borne 4-nonylphenol and 17 β-estradiol exposures during parr-smolt transformation on growth and plasma IGF-I of Atlantic salmon (*Salmo salar* L.). Aquat. Toxicol. 66: 255-265.

Baldwin, D.H., Spromberg, J.A., Collier, T.K. and Scholz, N.L. 2009. A fish of many scales: extrapolating sublethal pesticide exposures to the productivity of wild salmon populations. Ecol. Appl. 19: 204-2015.

Barton, A. 2000. Salmonid fishes differ in their cortisol and glucose responses to handling and transport stress. North Amer. J. Aquaculture 62: 12-18.

Baskaran S., Kookana R.S., Naidu R. 1997. Determination of the insecticide imidacloprid in water and soil using high-performance liquid chromatography. J. Chromatogr A. 787: 271-275.

Bolly, P., Rees, B.B. and Williamson, L.A.C. 2007. Vertebrate osmoregulation: a student laboratory exercise using teleost fish. Adv. Physiol. Educ. 31: 352-357.

Burridge, L., Weis, J., Cabello, F., Pizarro, J. and Bostick, K. 2010. Chemical use in salmon aquaculture: a review of current practices and possible environmental effects. Aquaculture 306: 1-23.

Busacker, G.P., Adelman, I.R. and Goolish, E.M. 1990. Growth. In: Schreck, C.B. and Boyle, P.B. (Eds.), Methods for Fish Biology. American Fisheries Society, USA. pp. 363-364.

CFIA (Canadian Food Inspection Agency). 2002. Determination of EBDC in Fruits and Vegetables (HPLC with Fluorescence Detection Method). Version SPR-002-V2.3, Canadian Food Inspection Agency, Calgary Laboratory. http://www-infocris.iaea.org/Download/SPR_002.pdf. (accessed online April 27, 2015)

CCME (Canadian Council of Ministers of the Environment). 1999a. Canadian water quality guidelines for the protection of aquatic life: Deltamethrin. In: Canadian environmental quality guidelines, 1999. Canadian Council of Ministers of the Environment, Winnipeg, MB.

CCME (Canadian Council of Ministers of the Environment). 1999b. Canadian water quality guidelines for the protection of aquatic life: Glyphosate. In: Canadian environmental quality guidelines, 1999. Canadian Council of Ministers of the Environment, Winnipeg, MB.

CCME (Canadian Council of Ministers of the Environment). 2007. Canadian water quality guidelines for the protection of aquatic life: Imidacloprid. In: Canadian environmental quality guidelines, 1999. Canadian Council of Ministers of the Environment, Winnipeg, MB.

Ceyhun, S.B., Şentürk, M., Ekinci, D., Erdoğan, O., Çiltaş, A. and Kocaman, E.M. 2010. Deltamethrin attenuates antioxidant defense system and induces the expression of heat shock protein 70 in rainbow trout. Comp. Biochem. Physiol. Part C 152: 215-223.

DuBeau, S.F., Pan, F., Tremblay, G.C. and Bradley, T.M. 1998. Thermal shock of salmon in vivo induces the heat shock protein hsp 70 and confers protection against osmotic shock. Aquaculture 168: 311-323.

Eder, K.J., Leutenegger, C.M., Köhler, H.-R. and Werner, I. 2009. Effects of neurotoxic insecticides on heat-shock proteins and cytokine transcription in Chinook salmon (*Oncorhynchus tshawytscha*). Ecotoxicol. Environ. Safety 72: 182-190.

Ellman, G.L., Courtney, K.D., Andres, Jr., V. and Featherstone, R.M. 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol. 7: 88-95.

Fairchild, W.L., Swansburg, E.O., Arsenault, J. T. and Brown, S.B. 1999. Does an association between pesticide use and subsequent declines in catch of Atlantic salmon (*Salmo salar*) represent a case of endocrine disruption? Environ. Health Perspect. 107 (5): 349-357.

Fairchild, W.L., Brown, S.B. and Moore, A. 2002. Effects of freshwater contaminants on marine survival in Atlantic salmon. NPAFC Tech. Rep. 4: 30-32.

Ferrari, A., Venturino, A. and Pechén de D'Angelo, A.M. 2004. Time course of brain cholinesterase inhibition and recovery following acute and subacute azinphosmethyl, parathion, and carbaryl exposure in the goldfish (*Carassius auratus*). Ecotox. Environ. Safety 57: 420-425.

Ferrari, A., Venturino, A. and Pechén de D'Angelo, A.M. 2007. Muscular and brain cholinesterase sensitivities to azinphos methyl and carbaryl in the juvenile rainbow trout *Oncorhynchus mykiss*. Comp. Biochem. Physiol. Part C 146: 308-313.

Harris, K.A., N. Dangerfield, M. Woudneh, T. Brown, S. Verrin and P.S. Ross. 2008. Partitioning of Current-Use and Legacy Pesticides in Salmon Habitat in British Columbia, Canada. Environ. Toxicol. Chem. 27: 2253–2262.

Hartwell, S.I. 2011. Chesapeake Bay watershed pesticide use delines but toxicity increases. Environ. Toxicol. Chem. 30:1223-1231.

Hoar, W.S. 1988. The physiology of smolting salmonides. In: Hoar, W.S., Randall, D.J. (Eds.), Fish Physiology, vol. XI. The physiology of developing fish, part B. Viviparity and posthatching juveniles. Academic Press, USA. pp. 275-343.

Iwama, G.K., Vijayan, M.M., Forsyth, R.B. and Ackerman, P.A. 1999. Heat shock proteins and physiological stress in fish. Amer. Zool. 39: 901-909.

Iwama, G.K., Afonso, L.O.B., Todgham, A., Ackerman, P. and Nakano, K. 2004. Are hsps suitable for indicating stressed states in fish? J. Exp. Biol. 207: 15-19.

Jarrard, H.E., Delaney, K.R. and Kennedy, C.J. 2004. Impacts of carbamate pesticides on olfactory neurophysiology and cholinesterase activity in coho salmon (*Oncorhynchus kisutch*). Aquat. Toxicol. 69: 133-148.

Keen, P.L., Higgs, D.A., Hall, K.J. and Ikonomou, M. 2005. Effects of dietary exposure of 4-nonylphenol on growth and smoltification of juvenile coho salmon (*Oncorhynchus kisutch*). Sci. Tot. Environ. 349: 81-94.

Kroglund, F., B.O. Rosseland, H.-C. Teien, B. Salbu, T. Kristensen and Finstad, B. 2008. Water quality limits for Atlantic salmon (Salmo salar L.) exposed to short term reductions in pH and increased aluminum simulating episodes. Hydrol. Earth Syst. Sci. 12:491-507.

Laetz, C.A., Baldwin, D.H., Collier, T.K., Hebert, V., Stark, J.D. and Scholz, N.L. 2009. The synergistic toxicity of pesticide mixtures: Implications for risk assessment and the conservation of endangered Pacific salmon. Environ. Health Perspec. 117: 348-353.

Lewis, S., Handy, R. D., Cordi, B., Billinghurst, Z. and Depledge, M.H. 1999. Stress proteins (Hsp's): Methods of detection and their use as an environmental biomarker. Ecotoxicol. 8: 351-368.

Li, Z-H., Zlabek, V., Grabic, R., Li, P., Machova,J. and Velisek, J. 2010. Effects of exposure to sublethal propiconazole on the antioxidant defense system and Na^+, K^+-ATPase activity in brain of rainbow trout, *Oncorhynchus mykiss*. Aquat.Toxicol. 98: 297-303.

Labenia, J.S., Baldwin, D.H., French, B.L., Davis, J.W. and Scholz, N.L. 2007. Behavioral impairment and increased predation mortality in cutthroat trout exposed to carbaryl. Mar. Ecol. Prog. Ser. 329: 1-11.

Madsen, S.S., Skovbølling, S., Nielsen, C., and Korsgaard, B. 2004. 17-β Estradiol and 4-nonylphenol delay smolt development and downstream migration in Atlantic salmon, *Salmo salar*. Aquat. Toxicol. 68: 109-120.

McCormick, S.D. 1993. Methods for nonlethal gill biopsy and measurement of Na^+, K^+-ATPase activity. Can. J. Fish. Aquatic. Sci. 50: 656-658.

McCormick, S.D. 1995. Hormonal control of gill Na^+, K^+-ATPase and chloride cell function. In: Wood, C.M. and Shuttleworth, T.J. (eds.), Fish Physiology, Vol.

XIV, Ionoregulation: Cellular and Molecular Approaches. New York: Academic Press. pp. 285-315.

McCormick, S.D., Hansen, L.P., Quinn, T.P. and Saunders, R.L. 1998. Movement, migration, and smolting of Atlantic salmon (*Salmo salar*). Can. J. Fish. Aquat. Sci. 55: 77-92.

McCormick, S.D., Shrimpton, J.M., Moriyama, S. and Björnsson, B.Th. 2002. Effects of an advanced temperature cycle on smolt development and endocrinology indicate that temperature is not a zeitgeber for smelting in Atlantic salmon. J. Exp. Biol. 205: 3553-3560.

McCormick, S.D., Lerner, D.T., Monette, M.Y., Nieves-Puigdoller, K., Kelly, J.T. and Björnsson, B.T. 2009. Taking It with You When You Go: How Perturbations to the Freshwater Environment, Including Temperature, Dams, and Contaminants, Affect Marine Survival of Salmon. Amer. Fish. Soc. Symp. 69: 195–214

McCormick, S.D., Lerner, D.T., Regish, A.M., O'Dea, M.F. and Monette, M.Y. 2012. Thresholds for short-term acid and aluminum impacts on Atlantic salmon smolts. Aquaculture 362-363: 224-231.

Miller, G.L. 1959. Protein determination for large numbers of samples. Anal. Chem. 31: 964.

Moore, A., Scott, A.P., Lower, N., Katsiadaki, I. and Greenwood, L. 2003. The effects of 4-nonylphenol and atrazine on Atlantic salmon (*Salmo salar* L) smolts. Aquaculture 222: 253-263.

Moore, A., Lower, N., Mayer, I. and Greenwood, L. 2007. The impact of a pesticide on migratory activity and olfactory function in Atlantic salmon (*Salmo salar* L) smolts. Aquaculture 273: 350-359.

Moore, A., Cotter, D., Quayle, V., Rogan, G., Poole, R., Lower, N. and Privitera, L. 2008. The impact of a pesticide on the physiology and behaviour of hatchery-reared Atlantic salmon, *Salmo salar*, smolts during the transition from fresh water to the marine environment. Fish. Manag. Ecol. 15: 385-392.

Morgan, M.J., Fancey, L.L. and Kiceniuk, J.W. 1990. Response and recovery of brain acetylcholinesteraseivity in Atlantic salmon (*Salmo salar*) exposed to fenitrothion. Can. J. Fish. Aquat.Sci. 47: 1652-1654.

Nieves-Puigdoller, K., Björnsson, B.T. and McCormick, S.D. 2007. Effects of hexazinone and atrazine on the physiology and endocrinology of smolt development in Atlantic salmon. Aquat. Toxicol. 84: 27-37.

Pérez-Casanova, J.C., Hamoutene, D., Volkoff, D., Mabrouk, G., Samuelson,S. and Burt, K. 2010. Effects of injection of lipopolysaccharides from *Aeromonas salmonicida* on some aspects of cod (*Gadus morhua*) immunity and appetite hormones. Can. Tech. Rep. Fish. Aquat. Sci. 2878: v + 11 p.

Rendell, J.L., Fowler, S., Cockshutt, A. and Currie, S. 2006. Development-dependent differences in intracellular localization of stress proteins (hsps) in rainbow trout, *Oncorhynchus mykiss*, following heat shock. Comp. Biochem. Physiol. D. 1: 238-252.

Ross, P.S., Kennedy, C.J., Shelley, L.K., Tierney, K.B., Patterson, D.A., Fairchild, W.L. and Macdonald, R.W. 2013. The trouble with salmon: relating pollutant exposure to toxic effect in species with transformational life histories and lengthy migrations. Can. J. Fish. Aquat. Sci. 70:1252-1264.

Sanders, B.M. 1993. Stress proteins in aquatic organisms: an environmental perspective. Critical Reviews in Toxicology 23: 49-75.

Shelley, L.K., Balfry, S.K., Ross, P.S. and Kennedy, C.J. 2009. Immunotoxicological effects of a sub-chronic exposure to selected current-use pesticides in rainbow trout (*Oncorhynchus mykiss*). Aquat. Toxicol. 92: 95-103.

Silva de Assis, H.C., Nicareta, L., Salvo, L.M., Klemz, C., Truppel, J.H. and Calegari, R. 2009. Biochemical biomarkers of exposure to deltamethrin in freshwater fish, *Ancistrus multispinis*. Braz. Arch. Biol. Technol. 52: 1-9.

Stone, W.W., Robert J.G. and K.R. Ryberg. 2014. Pesticides in U.S. Streams and Rivers: Occurrence and Trends during 1992–2011. Environ. Sci. Technol. 48: 11025–11030.

Struger, J. and Fletcher, T. 2007. Occurrence of lawn care agricultural pesticides in Don River and Humber River watersheds (1998-2002). J. Great Lakes Res. 33: 887-905.

Struger, J., Thompson, D., Staznik, B., Martin, P., McDaniel, T. and Martin, C. 2008. Occurrence of glyphosate in surface waters in southern Ontario. Bull. Environ. Contam. Toxicol. 80: 378-384.

Suvetha, L., Ramesh, M. and Saravanan, M. 2010. Influence of cypermethrin toxicity on ionic regulation and gill Na^+, K^+-ATPase activity of a freshwater teleost fish *Cyprinus carpio*. Environ. Toxicol. Pharmacol. 29: 44-49.

Thorstad, E.B., Whoriskey, F., Uglem, I., Moore, A., Rikardsen, A.H. and Finstad, B. 2012. A critical life stage of the Atlantic salmon *Salmo salar*: behaviour and survival during the smolt and initial post-smolt migration. J. Fish Biol. 81: 500-542.

Thorstad, E.B., Uglem, I., Finstad, B., Kroglund, F., Einarsdottir, I.E., Kristensen, T., Diserud, O., Arechavala-Lopez, P., Mayer, I., Moore, A., Nilsen, R., Bjornsson, B.T. and Okland, F. 2013. Reduced marine survival of hatchery-reared Atlantic salmon post-smolts exposed to aluminium and moderate acidification in freshwater. Est. Coast. Shelf Sci. 124: 34-43.

Tomlin, C.D.S. (ed.). 1997. The pesticide manual: a world compendium, 11th edition. British Crop Protection Council and Royal Society of Chemistry, Surrey, UK.

U.S. EPA. 2007. Risks of mancozeb and maneb uses to the federally listed California red legged frog (*Rana aurora draytonii*). Pesticide Effects Determination, Environmental Fate and Effects Division, Office of Pesticide Programs, Washington, D.C.

Waring, C.P. and Moore, A. 2004. The effect of atrazine on Atlantic salmon (*Salmo salar*) smolts in fresh water and after seawater transfer. Aquat. Toxicol. 66: 93-104.

Yoshida, Y., Murakami, M., Murakami, T., Fujimoto, H., Takeda, K., Suzuki, S. and Hari, M. 2007. Determination of nonylphenol ethoxylates and octylphenol ethoxylates in environmental sample using 13C-labelled surrogate compounds. J. Air and Waste Manage. Assoc. 57: 1164-1171

Zaugg, W.S. 1982. A simplified preparation for adenosine triphosphatase determination in gill tissue. Can. J. Fish. Aquat. Sci. 39: 215-217.

Zinkl, J.G., Shea, P.J., Nakamoto, R.J. and Callman, J. 1987. Effects of cholinesterases of rainbow trout exposed to acephate and methamidophos. Bull. Environ. Contam. Toxicol. 38: 22-28.

Table 1. Difference between mean control SGRW and treatment SGRW. Asterisks represent significant difference (* $P < 0.05$, ** $P < 0.005$) (Dunnett's test).

Treatment	July	September	November
Cypermethrin 0.75 µg/L		*	
Cypermethrin 0.15 µg/L			
Cypermethrin 0.03 µg/L			
Deltamethrin 2.0 µg/L			
Deltamethrin 0.59 µg/L		*	
Deltamethrin 59 ng/L			
Imidacloprid 90 mg/L	*		
Imidacloprid 8.6 mg/L			
Imidacloprid 10 µg/L	*		
Glyphosate 3000 µg/L			
Glyphosate 650 µg/L	*	*	
Glyphosate 65 µg/L	*		
Mancozeb 1500 µg/L			
Mancozeb 150 µg/L			
Mancozeb 50 µg/L			
Nonylphenol 20 µg/L			

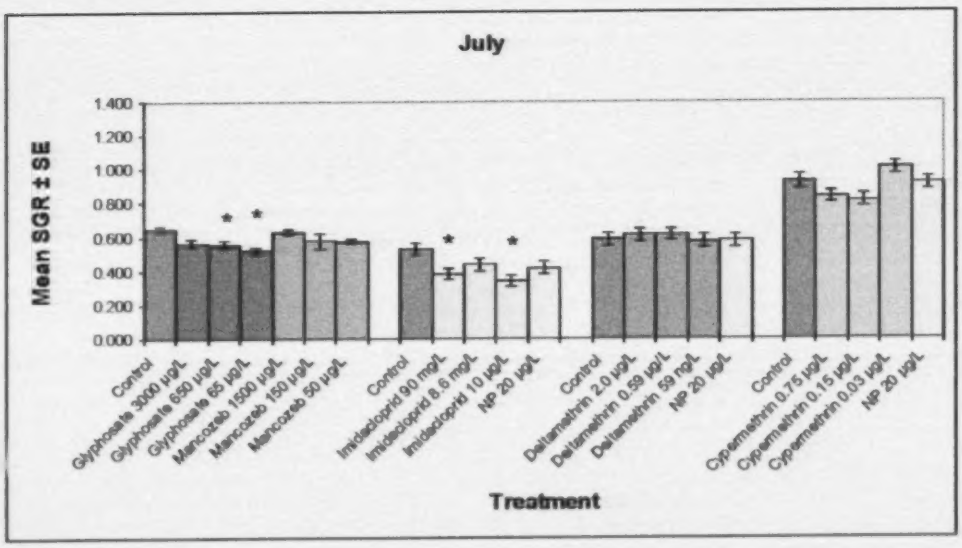
Table 2. Difference between frequency distributions of control SGRW and treatment SGRW. Asterisks represent significant difference (* $P < 0.05$, ** $P < 0.005$) (KS test).

Treatment	July	September	November
Cypermethrin 0.75 µg/L	*	**	
Cypermethrin 0.15 µg/L		*	
Cypermethrin 0.03 µg/L			
Deltamethrin 2.0 µg/L			
Deltamethrin 0.59 µg/L		*	
Deltamethrin 59 ng/L		*	
Imidacloprid 90 mg/L	*		*
Imidacloprid 8.6 mg/L	*		
Imidacloprid 10 µg/L	**	*	
Glyphosate 3000 µg/L	*		
Glyphosate 650 µg/L	*	*	
Glyphosate 65 µg/L	**		
Mancozeb 1500 µg/L			
Mancozeb 150 µg/L			
Mancozeb 50 µg/L	*		
Nonylphenol 20 µg/L	** (for 2008)	* (for 2008 & 2010)	

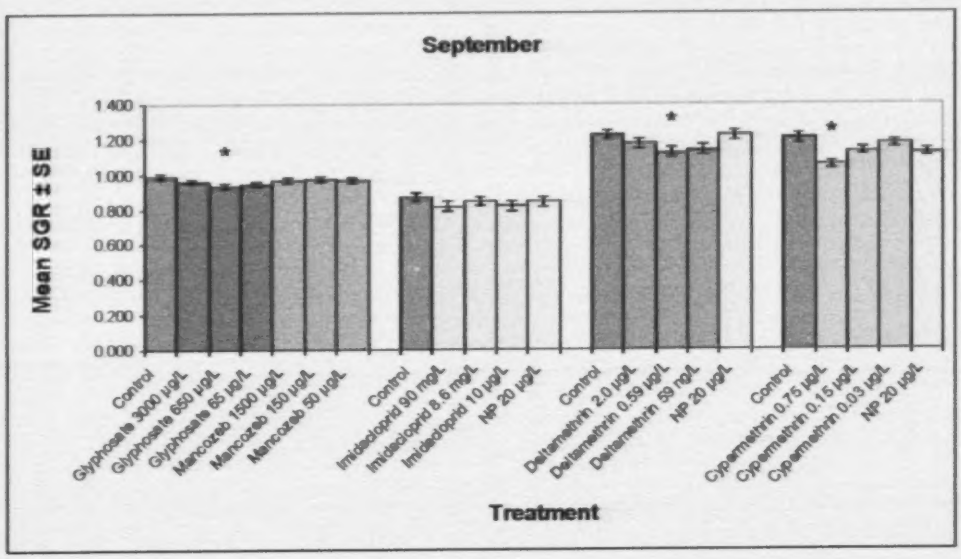
Table 3. Water analysis - nominal and measured concentrations (data represent mean value ± SE). *average of only two analyses; ** Contract lab encountered analytical problems and no concentrations were determined.

Treatment	Measured Conc. ± SE
Cypermethrin 0.75 µg/L	0.559 µg/L ±0.011
Cypermethrin 0.15 µg/L	0.134 µg/L ±0.007
Cypermethrin 0.03 µg/L	0.025 µg/L ±0.010
Deltamethrin 2.0 µg/L	1.758 µg/L ±0.086
Deltamethrin 0.59 µg/L	0.634 µg/L ±0.032
Deltamethrin 0.059 µg/L	0.027 µg/L *
Imidacloprid 90 mg/L	Not available**
Imidacloprid 8.6 mg/L	Not available
Imidacloprid 10 µg/L	Not available
Glyphosate 3000 µg/L	1635 µg/L ± 129
Glyphosate 650 µg/L	714 µg/L ± 114
Glyphosate 65 µg/L	32 µg/L ± 5
Mancozeb 1500 µg/L	653 µg/L ± 108
Mancozeb 150 µg/L	130 µg/L ± 70
Mancozeb 50 µg/L	42 µg/L ± 19
Nonylphenol 20 µg/L	7.37 µg/L ± 0.94

a)


July
Mean SGR ± SE
1.400
1.200
1.000
0.800
0.600
0.400
0.200
0.000
Control
Glyphosate 3000 µg/L
Glyphosate 650 µg/L
Glyphosate 65 µg/L
Mancozeb 1500 µg/L
Mancozeb 150 µg/L
Mancozeb 50 µg/L
Control
Imidacloprid 90 mg/L
Imidacloprid 8.6 mg/L
Imidacloprid 10 µg/L
NP 20 µg/L
Control
Deltamethrin 2.0 µg/L
Deltamethrin 0.59 µg/L
Deltamethrin 59 ng/L
NP 20 µg/L
Control
Cypermethrin 0.75 µg/L
Cypermethrin 0.15 µg/L
Cypermethrin 0.03 µg/L
NP 20 µg/L
Treatment

b)


September
Mean SGR ± SE
1.400
1.200
1.000
0.800
0.600
0.400
0.200
0.000
Control
Glyphosate 3000 µg/L
Glyphosate 650 µg/L
Glyphosate 65 µg/L
Mancozeb 1500 µg/L
Mancozeb 150 µg/L
Mancozeb 50 µg/L
Control
Imidacloprid 90 mg/L
Imidacloprid 8.6 mg/L
Imidacloprid 10 µg/L
NP 20 µg/L
Control
Deltamethrin 2.0 µg/L
Deltamethrin 0.59 µg/L
Deltamethrin 59 ng/L
NP 20 µg/L
Control
Cypermethrin 0.75 µg/L
Cypermethrin 0.15 µg/L
Cypermethrin 0.03 µg/L
NP 20 µg/L
Treatment

c)

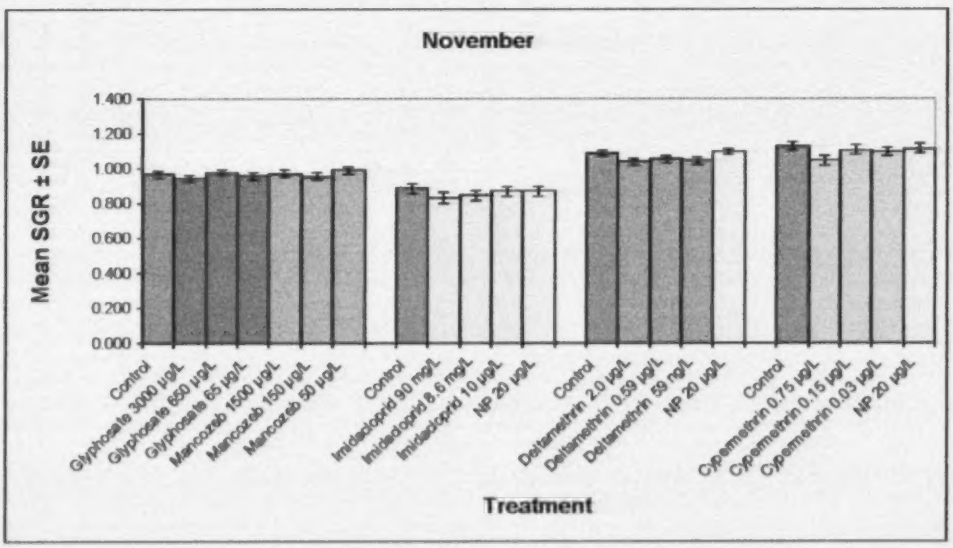


Figure 1a-c. Mean SGRW and standard errors of control, NP and pesticide treated fish (July, September and November). Asterisks indicate significant differences from controls ($P < 0.05$) (Dunnett's test).

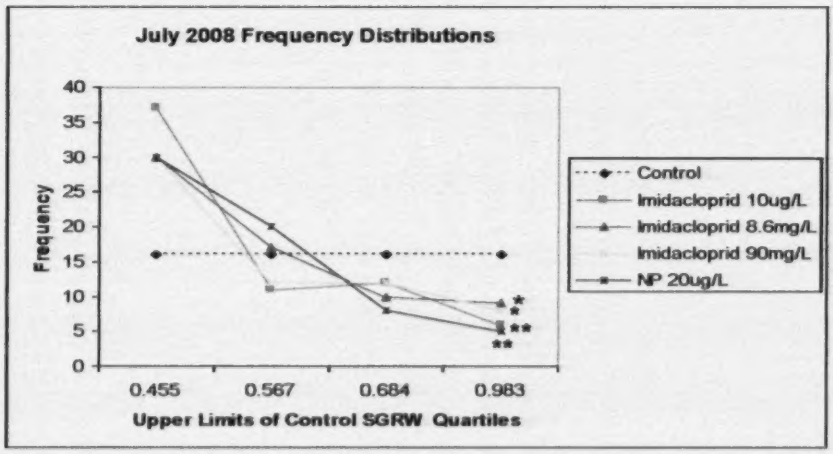


Figure 2. July SGRW frequency distributions of controls, NP and imidacloprid treated smolts. Tick marks on the X-axis are the upper-limit of each control SGRW quartile. Y-axis gives the frequency defined by the control SGRW quartiles. Asterisks represent significant difference from the control SGRW frequency distribution (* $P < 0.05$, ** $P < 0.005$) (KS test).

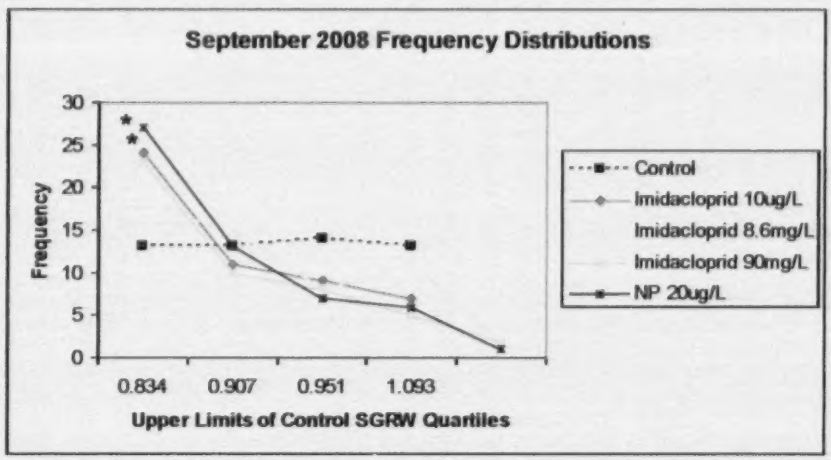


Figure 3. September SGRW frequency distributions of controls, NP and imidacloprid treated smolts. Tick marks on the X-axis are the upper-limit of each control SGRW quartile. Y-axis gives the frequency defined by the control SGRW quartiles. Asterisks represent significant difference from the control SGRW frequency distribution (* $P < 0.05$, ** $P < 0.005$) (KS test).

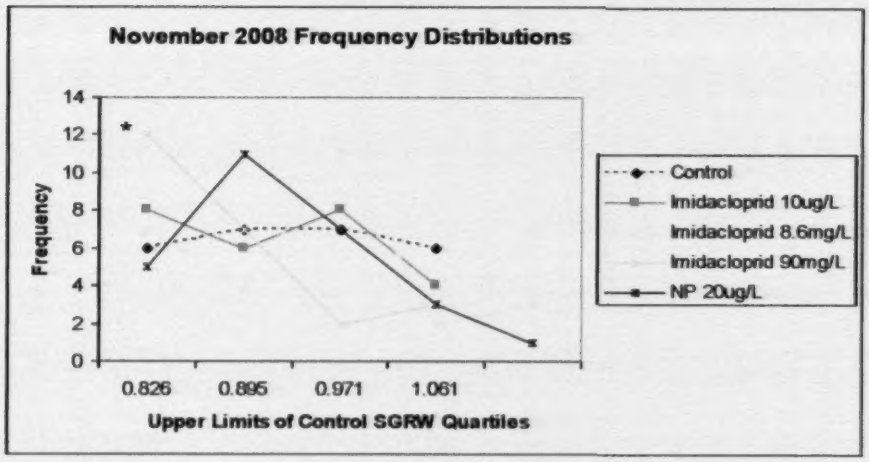


Figure 4. November SGRW frequency distributions of controls, NP and imidacloprid treated smolts. Tick marks on the X-axis are the upper-limit of each control SGRW quartile. Y-axis gives the frequency defined by the control SGRW quartiles. Asterisks represent significant difference from the control SGRW frequency distribution (* $P < 0.05$, ** $P < 0.005$) (KS test).

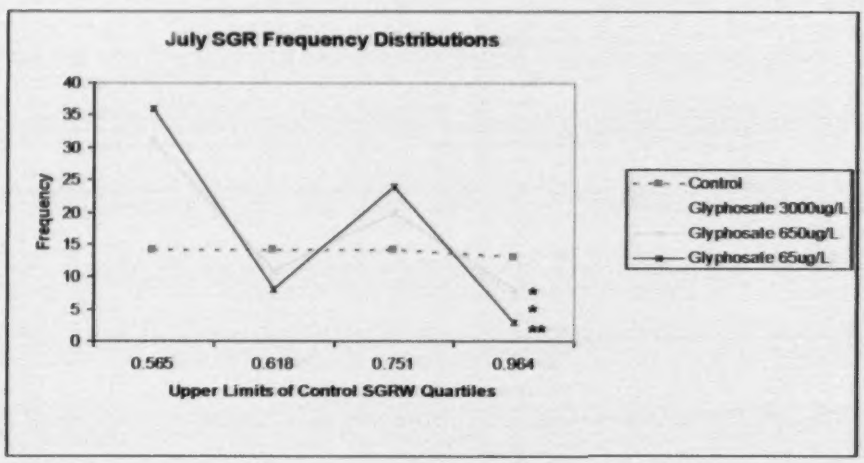


Figure 5. July SGRW frequency distributions of controls and glyphosate treated smolts. Tick marks on the X-axis are the upper-limit of each control SGRW quartile. Y-axis gives the frequency defined by the control SGRW quartiles. Asterisks represent significant difference from the control SGRW frequency distribution (* $P < 0.05$, ** $P < 0.005$) (KS test).

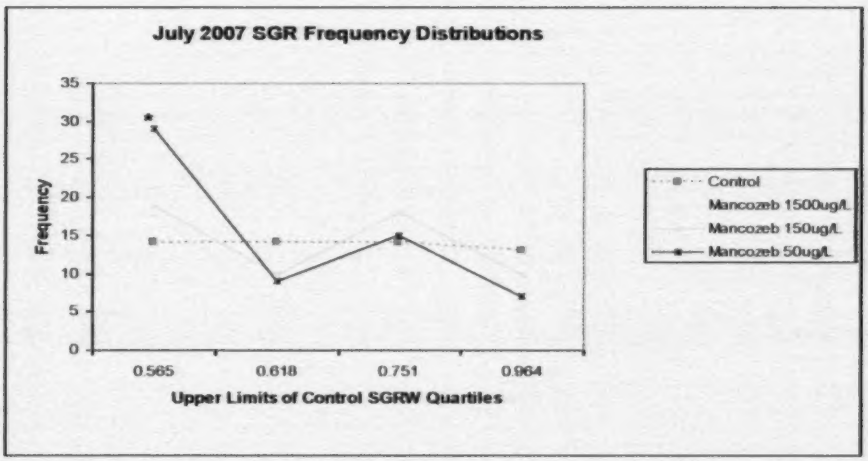


Figure 6. July SGRW frequency distributions of controls and mancozeb treated smolts. Tick marks on the X-axis are the upper-limit of each control SGRW quartile. Y-axis gives the frequency defined by the control SGRW quartiles. Asterisks represent significant difference from the control SGRW frequency distribution (* $P < 0.05$, ** $P < 0.005$) (KS test).

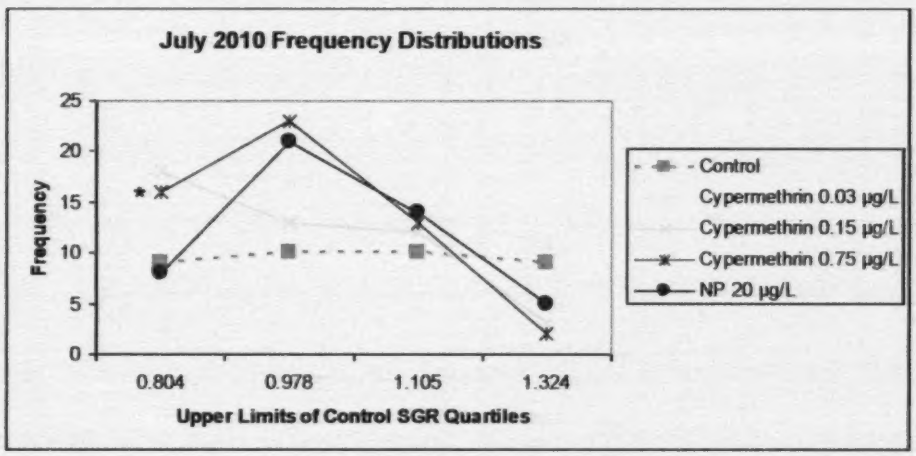


Figure 7. July SGRW frequency distributions of controls, cypermethrin and NP treated smolts. Tick marks on the X-axis are the upper-limit of each control SGRW quartile. Y-axis gives the frequency defined by the control SGRW quartiles. Asterisks represent significant difference from the control SGRW frequency distribution (* $P < 0.05$, ** $P < 0.005$) (KS test).

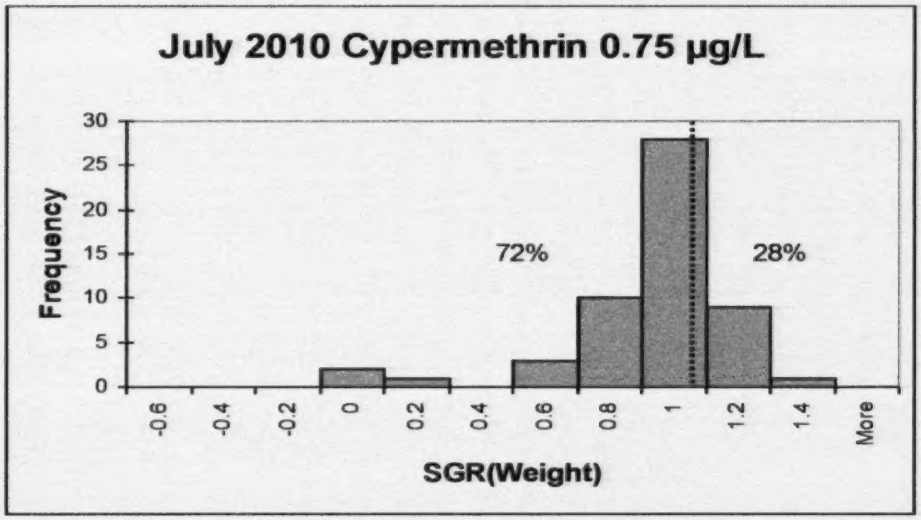


Figure 8. July SGRW frequency histogram of cypermethrin treated smolts. Dotted line represents the median SGRW (i.e. 0.978) of controls.

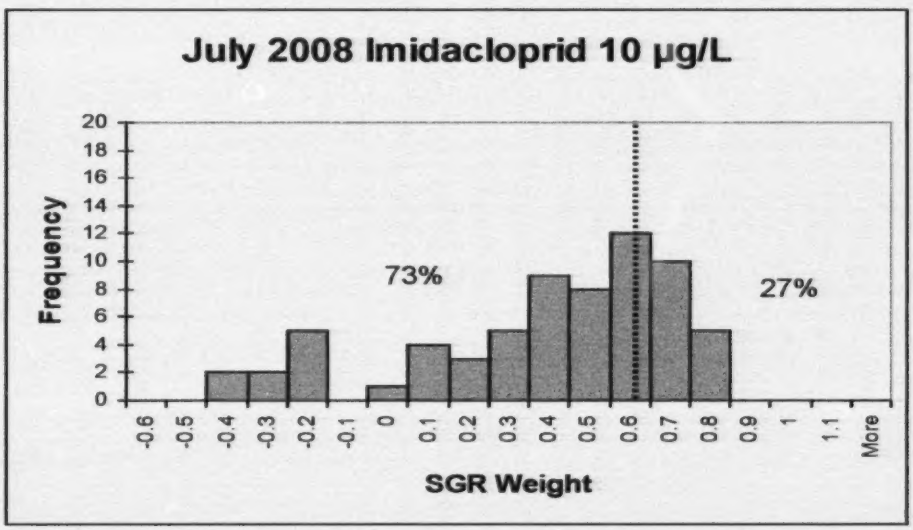


Figure 9. July SGRW frequency histogram of imidacloprid treated smolts. Dotted line represents the median SGRW (i.e. 0.567) of controls.

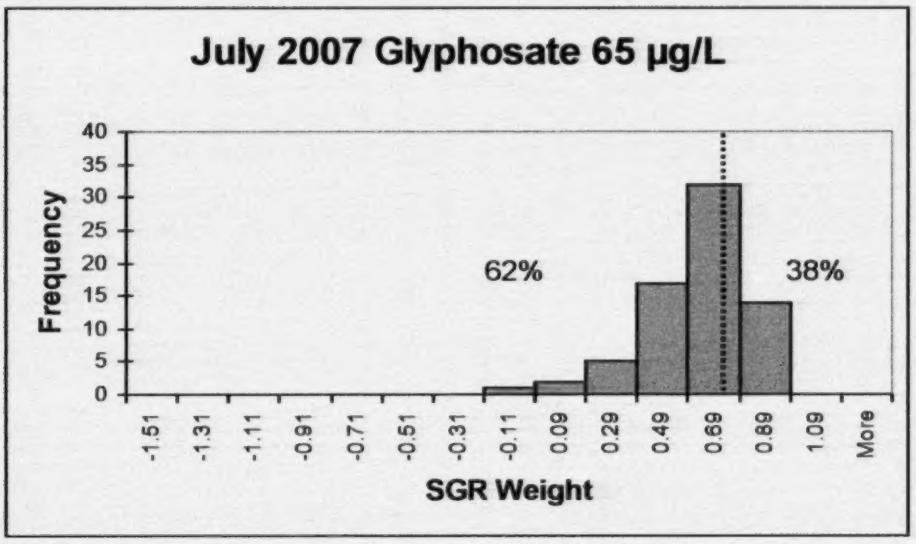


Figure 10. July SGRW frequency histogram of glyphosate treated smolts. Dotted line represents the median SGRW (i.e. 0.618) of controls.

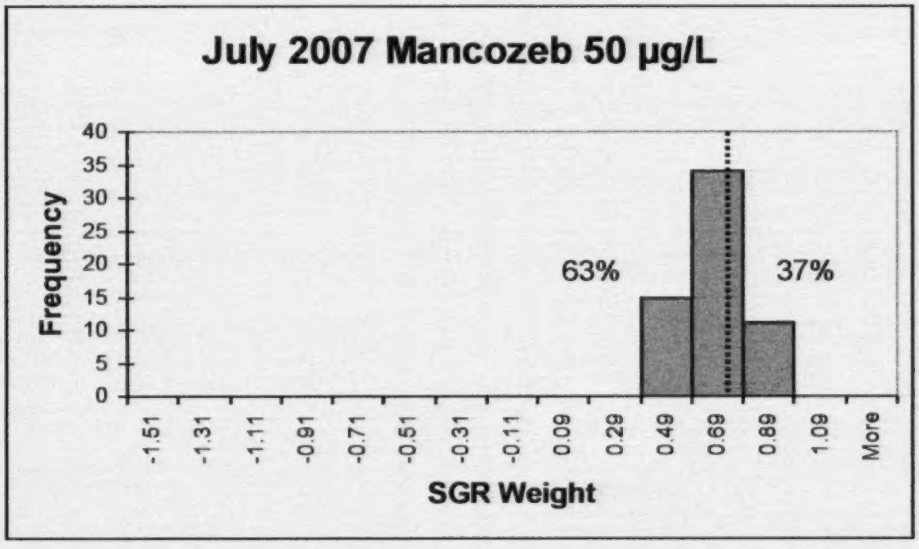


Figure 11. July SGRW frequency histogram of mancozeb treated smolts. Dotted line represents the median SGRW (i.e. 0.618) of controls.

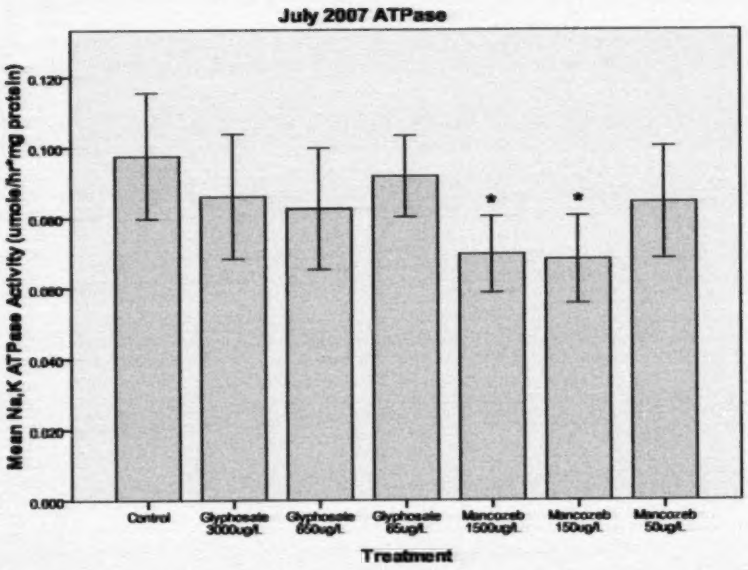


Figure 12. Mean Na, K, ATPase and standard errors of control and pesticide treated fish. Asterisks indicate significant differences from controls (Dunnett's) ($P < 0.05$).

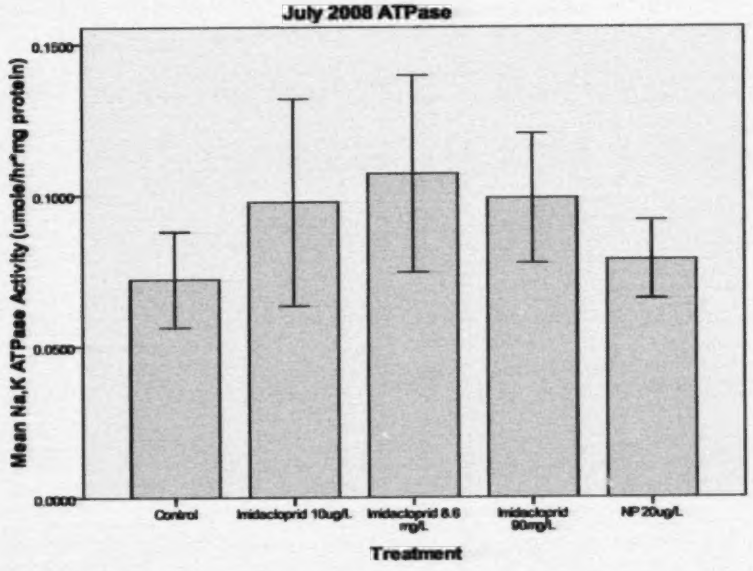


Figure 13. Mean Na, K, ATPase and standard errors of control, NP and pesticide treated fish. Asterisks indicate significant differences from controls ($P < 0.05$) (Dunnett's).